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Hyper Sensitive Strip Test with Chemi-luminescence Signal Band

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Abstract

A simple and sensitive method using lateral flow immunoassay (LF) using chemiluminescence reaction has been developed for the detection of antigen. In this work, horseradish peroxidase (HRP) samples were used to confirm the validity of this method and human chorionic gonadotropin (hCG) samples were used to evaluate the limit of detection (LOD) of this method. Through HRP test, this method was confirmed to be reliable for immunoassay, and the results of hCG test show that this method was very sensitive compared with conventional strip test using color of gold nanoparticles or latex beads.

Keywords: Lateral Flow Immunoassay, Chemiluminescence, HRP, hCG

1. Introduction

Lateral flow immunoassay (LF) has been widely used for the detection of biomarkers for diseases[1], microorganisms[2] and toxins[3]. Recently, the discoveries in genomics, functional genomics, and proteomics have been reported new biomarkers for medical diagnosis, which require of far lower detection limits in comparison with that of the conventional LF methods of approximately 1 -10 ng/ml[4]. To improve the sensitivity of LF method, various kinds of detection methods have been reported, such as image scanning[5], fluorescence labeled detection antibodies[6], conductivity measurement[7], impedance spectroscopy[8] and so on.

In this work, the chemiluminescence (CL) by the reaction between horseradish peroxidase (HRP) and luminol is applied for the improvement of the sensitivity of the LF method, and the test result is displayed by using a signal band with luminescence instead of the color change. As the sensitivities of chromogenic reaction by color change, fluorescence and CL are usually known to be 10^{-9}, 10^{-12}, and 10^{-15}, respectively[9], the CL-based signal band was expected to be improve the sensitivity of the LF method. Because of such a high sensitivity, the chemiluminescence (CL) method has widely been used as a versatile, sensitive tool in the field of biotechnology, pharmacology, molecular biology and clinical and environmental assays.

2. Chemiluminescence reaction

A sensible mechanism of the reaction is schematically presented in Fig. 1. Base removes the nitrogen protons leaving a negative charge which moves onto the carbonyl oxygen to form what is known as an enolate. HRP next catalyze cleavage of peroxide and the oxygen from the peroxide performs a cyclic addition to the two (previously) carbonyl carbons. Nitrogen is an excellent leaving group because its own bonds are so strong (and as a gas, it is

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entropically favoured too) so the charge on the oxygens come back down to form carboxylate anions by expelling nitrogen gas. This leaves 3-APA* and this excited form of luminol is stabilized and gives an emission spectra.

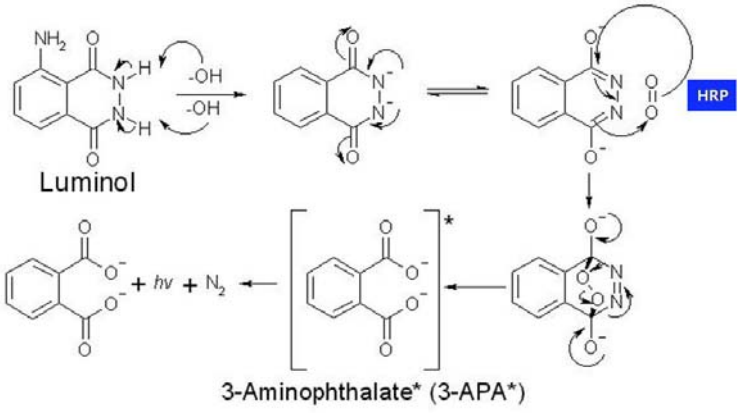

Fig. 1. Chemiluminescence reaction of Luminol catalyzed by HRP

3. Detection of HRP samples

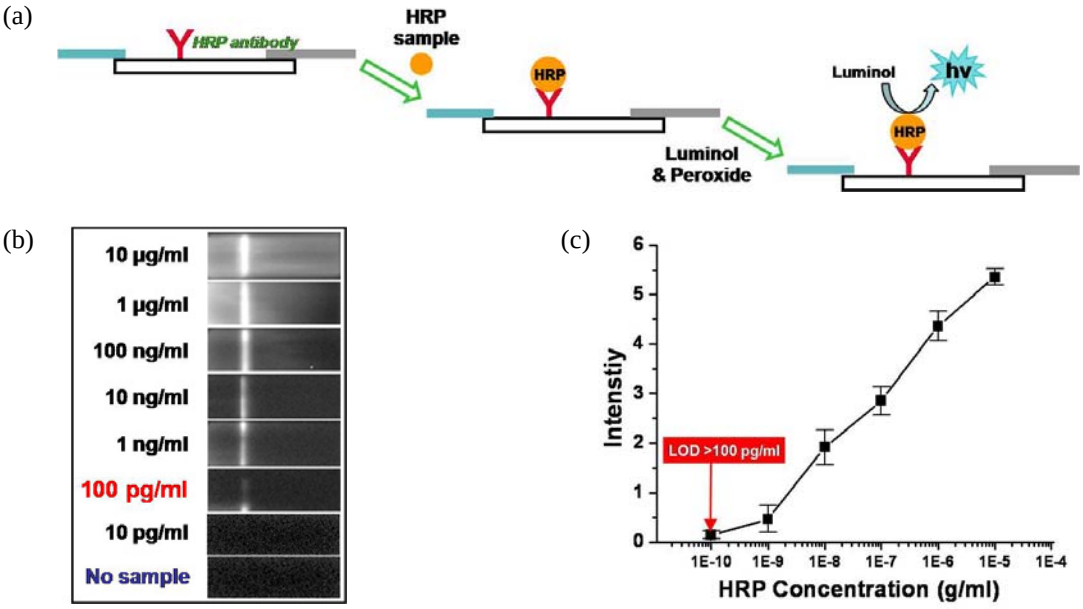

Fig. 2. Strip test with a luminescence signal band for the detection of HRP. (a) Assay format; (b) Images of signal bands with luminescence; (c) Quantitative correlation between signal intensity

In order to demonstrate a signal band with luminescence by the lateral flow immunoassay method, HRP was used as a model analyte. Fig. 2. (a) shows assay format of HRP detection. The signal band with luminescence was observed by using a CCD camera. (Fig. 2. (b)) For the comparison of the signal intensities among different concentration samples of Fig. 2. (b), the intensity was calculated by the intensity integration of the band region with the image analysis software. This result shows that this method was reliable. Also the signal band intensity and the concentration of HRP in log scale could be linearly correlated, so the quantitative analysis of a target analyte is feasible by using this method.

4. Detection of hCG samples

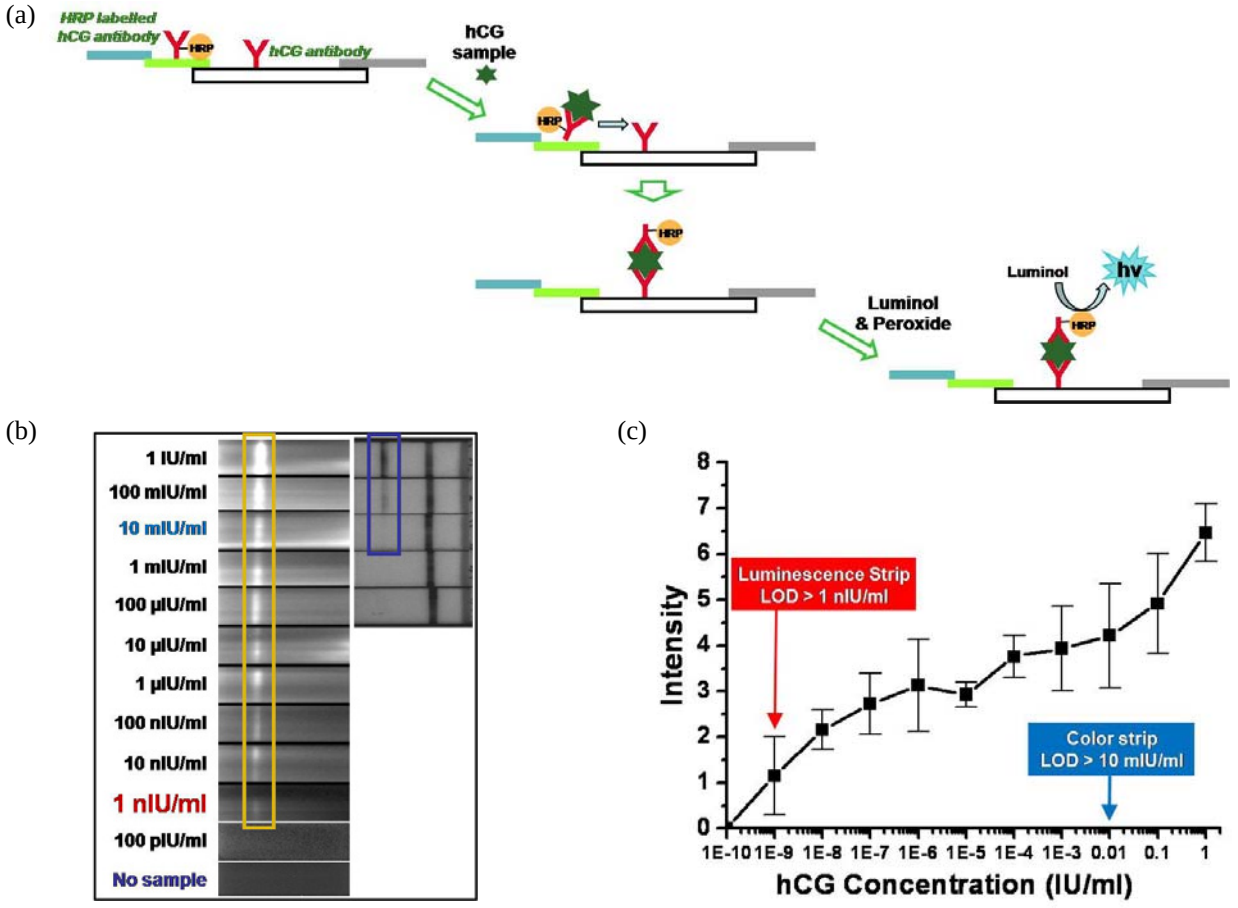

Fig. 3. hCG detection by using the strip test with a luminescence signal band. (a) Assay format. (b) Comparison signal bands between the luminescence strip test and the conventional test method. (c) Correlation curve between the strip test with luminescence and the conventional test

As a model of sandwich-type immunoassay which is generally used for the medical diagnosis of various diseases, the hCG test was selected to demonstrate the applicability demonstration of the strip test with a luminescence signal band. In the strip test for hCG, a conjugation pad with an immobilized anti-hCG antibodies coupled with HRP (Ab-hCG) is added to the previous test format as shown in Fig. 3. (a). As shown in Fig. 3. (b), the images of luminescence signal bands were obtained by using the documentation system and the LOD was estimated to be less than 1 nIU. Then this result was compared with the LOD of the conventional LF based on gold-nanoparticle. As shown in Fig. 3. (b), the conventional method could report the recognizable signal band at the concentration of more than 100 mIU. As shown in Fig. 3. (c), the detection at low concentration range can be significantly improved by using the strip test with luminescence signal band. This means that the strip test with luminescence signal band can improve the detection limit as much as 10^6-fold for the LF method based on a sandwich-type assay format.

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